Evidence for Conformational Changes in Euglena Chloroplasts [1, 2]

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Introduction

Spinach chloroplasts carry out several energy-dependent processes such as conformational changes and ion translocation, in addition to the synthesis of ATP. The evidence for energy-dependent conformational changes has come from light-scattering studies by Packer (11, 12), Jagendorf and Hind (5), and Dilley and Vernon (1). Itoh et al. (3) and Izawa et al. (4) have observed changes in chloroplast volume by Coulter counter studies, "chlorocrit" determinations, and in ultrastructure in vivo (6) and in vitro (3) by electron microscopy of illuminated spinach chloroplasts. Mukohata and Packer (8) and Ohnishi (10) have also reported light-dependent viscosity changes in suspensions of spinach chloroplasts. Packer and Marchant (13) and Ohnishi (10) have extracted contractile proteins from spinach chloroplasts. Conditions similar to those required for structural changes (13) also support a light-dependent uptake of calcium and phosphate in isolated spinach chloroplasts (9). Hence, it is concluded that chloroplasts in a higher plant such as spinach should be capable of regulating both their ion and water contents.

It seemed worthwhile, therefore, to consider the possibility of the occurrence of a similar phenomenon in algae. It has been found that *Euglena* chloroplasts show light-dependent packed volume changes and energy-dependent conformational changes as revealed by light-scattering studies.

Materials and Methods

Conditions of Culture. *Euglena gracilis,* strain z, was grown at 25° in Hutner's Medium at pH 3.3 to 3.5 (2) under constant illumination of 20 w warm-white fluorescent bulbs, at 500 ft-c. Constant agitation and aeration were provided by bubbling a mixture of 3 % CO_2 in air into a 6-liter cylindrical flask, which contained 4 liters of medium. The inoculum consisted of a 40 ml suspension of cells grown under the same conditions and in the same medium using 125 ml Erlenmeyer flasks without aeration.

Chloroplast Isolation. The following procedures were performed at 4°. Cells were harvested after 3 to 4 days by centrifugation in a continuous-flow centrifuge at 3000 × *g*. The cell pellet was collected in 30 to 40 minutes, resuspended in 40 ml of buffered sucrose (0.5 M sucrose–0.1 M Tris, pH 8.0) and were disrupted by a single passage through a French Pressure Cell at 5000 psi. The broken cell preparation was centrifuged at 200 × *g* for 5 minutes, the resulting pellet was treated 3 more times in the same fashion with sucrose-Tris, pH 8.0, and the supernatants were collected and pooled. These fractions were then centrifuged at 600 × *g* for 15 minutes and the chloroplast-containing residue was resuspended in sucrose-Tris, and the 200 × *g* and 600 × *g* centrifugation steps were repeated, and again saving the 600 × *g* fraction. The resuspended 600 × *g* pellet was then washed twice with Tris (20 mM, pH 8)-NaCl (35 mM) by centrifugation for 15 minutes at 600 × *g*. The final pellet contained 15 to 25 mg of chlorophyll (15).

Packed Volume Studies. The final 600 × *g* fraction was diluted with NaCl (35 mM)-Tris (20 mM, pH 8) to yield a final chlorophyll concentration of 0.1 or 0.2 mg/ml. Duplicate 2 ml aliquots of this suspension in Tris (20 mM, pH 8)-NaCl (35 mM) with additions as indicated in individual experiments were added to 3 ml graduated protein (or "chlorocrit") tubes. A 150 w tungsten photoflood type lamp was used as light source. After the individual treatments, the tubes were centrifuged at 1000 × *g* and 25° in either light (2000 ft-c) or in darkness using specially-constructed transparent lucite tube holders which fit into the trunnions of the clinical centrifuge. Packed volumes were estimated by measuring the pellets at the appropriate times. In any particular experiment the reproductibility was excellent; the variability between identical samples was approximately 5 %.

Light scattering (546 mμ) by chloroplast suspensions was measured at 90° in a Brice-Phoenix Light Scattering Photometer modified for recording as described by Packer (11). The low intensity of the green light used for the scattering measurements was near the minimum of the photosynthetic action spectrum. Increases in scattering intensity following treatment with actinic light (Wratten #26 filter) are expressed as percent changes of the initial scattering level. The temperature of the system was maintained at 25° ± 0.1 by circulating liquid around a jacketed 1 cm cuvette.

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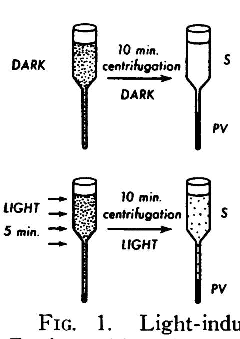
[4] Abbreviations: PMS, phenazine methosulfate; m-CCP, m-chloro-carbonyl cyanide phenylhydrazone: DCMU, 3-(3,4-dichlorophenyl)-1-1-dimethyl urea.



PV (ml)	Chlorophyll in S (mg/ml)	Protein (mg/ml)	Chlorophyll / Protein
0.220	0.036	0.60	0.060
0.140	0.075	1.20	0.062

FIG. 1. Light-induced packed volume changes in *Euglena* chloroplasts. Conditions as given in Methods; chlorophyll concentration was 0.2 mg/ml. Protein was determined by the method of Lowry et al. (7). PV = chloroplast packed volume; S = supernatant.

Results

Packed Volume Studies. Figure 1 shows that after preillumination and centrifugation in the light, the packed volume of chloroplasts is 36 % less than that for an identical suspension tested without illumination. The supernatant fractions of the light-treated samples contained about twice as much chlorophyll and protein as those from the dark, but the chlorophyll-protein ratio was the same in both. Furthermore, the shape of the absorption spectrum in the visible region was the same for both supernatant fractions and for a noncentrifuged chloroplast suspension. Spectral studies, chemical analysis, and microscopic examination revealed that some chloroplast fragments are present in the supernatant solutions and that these apparently sediment more slowly than the intact chloroplasts. A study of the packed volume changes as a function of centrifugation time (see control of fig 3) reveals that in the light the pellet volume is low after 3 minutes but becomes larger with increasing centrifugation time. In the dark the ability of the chloroplasts to sediment is quite different since after 3 minutes, it is generally impossible to measure any pellet volume. After 30 minutes of centrifugation, the particles of the supernatant fractions are completely sedimented and the light packed volume is still smaller than in the dark. When the chlorophyll concentrations are varied between 0.05 to 0.4 mg/ml, the packed volume after equilibrium was proportional to the chlorophyll concentration in the original suspension for centrifugation in the light and in the dark. Using this basic test system for "chlorocrit" determinations, a number of other factors affecting the packed volume were examined.

A study of the relative light intensity of packed volume changes showed that the threshold for the response lies between 250 and 500 ft-c. Also, light-mediated packed volume changes are temperature dependent since lowering the temperature abolishes the effect (fig 2A). Hence, a light-triggered, temperature-sensitive process is involved. This process is also influenced by the pH of the test system (fig 2B). Light-dependent packed volume changes are abolished

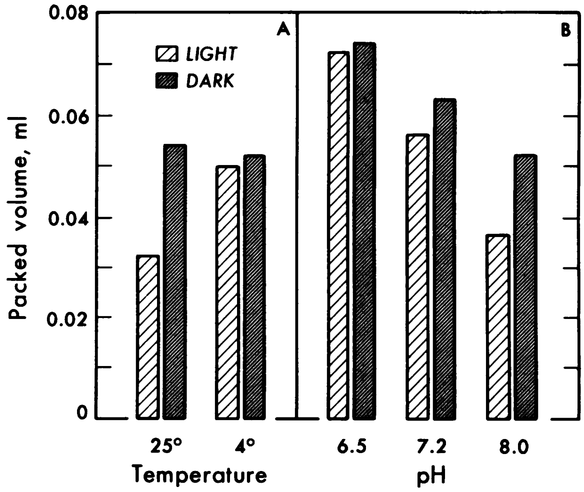


FIG. 2. Temperature and pH dependence of light-induced packed volume changes. The reaction mixture (2 ml) contained: A, NaCl (35 mM); Tris (20 mM, pH 8); and chloroplasts (0.1 mg chlorophyll/ml). B, Na^+ and K^+ phosphate buffer (0.067 M at indicated pH), and chloroplasts (0.2 mg chlorophyll/ml). Other conditions are as in figure 1.

by lowering the pH to 6.5. Also, the volume of the pellet becomes larger as the pH is lowered.

It has been found that photophosphorylation conditions as well as ATP (12) affected the conformational changes of spinach chloroplasts. The effect of ATP on the sedimentation and packed volume of chloroplasts is shown as a function of centrifugation time in figure 3. ATP elicited a decrease of the

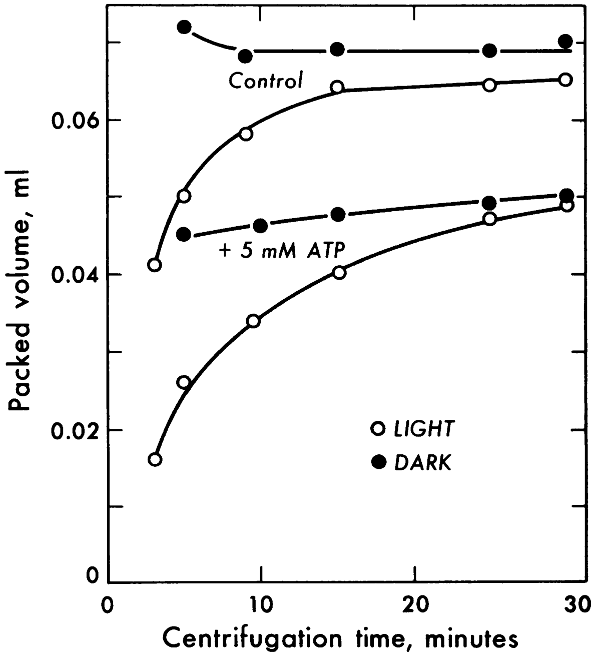


FIG. 3. Action of ATP on packed volume changes. Conditions as in figure 1; ATP added where indicated.

packed volume of the chloroplasts in either light or dark conditions as seen by the pellet volume after complete sedimentation. Apparently ATP does not affect the shape of the sedimentation curves either in the light or in the dark. Hence, its action would appear to be on decreasing chloroplast volume by about 25 to 30 %. Approximately the same values were obtained by Itoh et al. (3) for spinach chloroplasts.

As the effect of ATP was to reduce the packed volume both in the light and in the dark, it was predicted that in the presence of photophosphorylation reagents, the same response would occur. In fact, the addition of 2 mM ADP and 2 mM Pi elicits an alteration of the light sedimentation curves and decreases the pellet volume by 24 % after 4 minutes of centrifugation and 10 % after equilibrium was reached. In the dark no significant change was observed. The presence of 2 mM NH_4Cl in the same reaction mixture prevented the effect of ADP and Pi.

As noted in figures 1 and 3, the pattern of chloroplast sedimentation curves are remarkably different between light and dark; illuminated chloroplasts sediment much more slowly. Therefore, it was of interest to study this light effect by varying the period of the illumination between 0 and 30 minutes before starting the centrifugation. Readings were taken after centrifugation for 3 and 6 minutes. At these times, the chloroplasts are, of course, incompletely packed (see fig 1, 3). Figure 4 shows a very definite preillumination effect. At the shorter centrifugation time (3 min), there appears to be a direct relationship between preillumination time and packed volumes. With longer centrifugation time (6 min) the packed volumes increase and the effect of preillumination exposure begins to disappear; this is because the chloroplasts at 6 minutes are more completely sedimented.

Light Scattering Studies. These measurements have proved to be valuable in detecting conformational changes in chloroplasts (11). Figure 5 shows that actinic light induces a 16 to 18 % increase in the 90° light-scattering intensity of the chloroplast suspension incubated in the presence of certain cofactors. The effect is reversible and is considerably reduced in extent by 0.2 mM NH_4Cl and is virtually absent when the NH_4Cl concentration is increased to 2.2 mM. An examination of the requirements for these light-scattering changes and the action of several inhibitors is shown in table I. These data show that $MgCl_2$, PMS[4], and ascorbate are required for maximal scattering response, whereas P_i, ADP, and ATP apparently are not. Among the inhibitor substances tested, DCMU and antimycin *A* were found to be without effect at the concentrations employed. Scattering responses in the presence of 10 μM m-CCP and 2 mM NH_4Cl were 35 and 86 % less than that of the complete system respectively.

Unlike the case with spinach chloroplasts (11), ferricyanide alone did not support light-scattering changes. Adding ferricyanide to the PMS-ascorbate system (in excess of the ascorbate present) inhibited

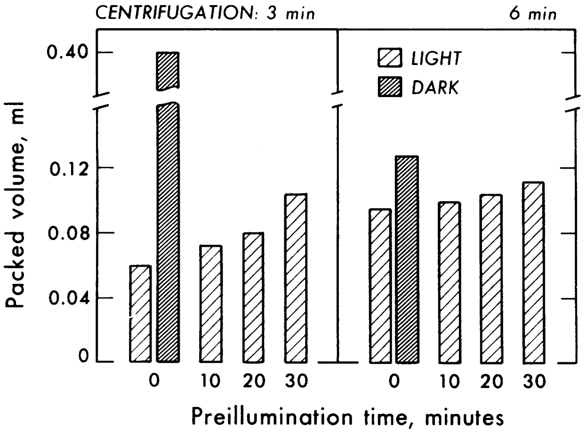


FIG. 4. Effect of preillumination on packed volume changes in *Euglena* chloroplasts. Conditions as in figure 1 except for preillumination and centrifugation time as shown.

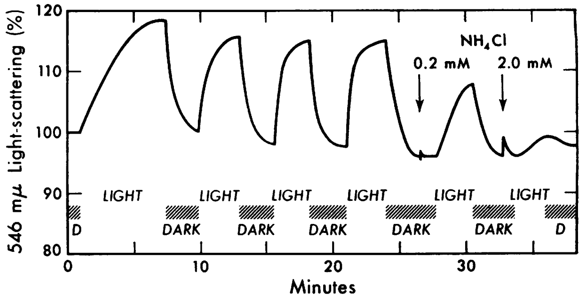


FIG. 5. Reversible scattering changes accompanying illumination of *Euglena* chloroplasts with actinic light. Conditions as in Methods. The reaction mixture (5 ml) contained: Tris (20 mM, pH 8), NaCl (35 mM), $MgCl_2$ (5 mM), ascorbate (2 mM), phenazine methosulfate (20 μM), and chloroplasts (5 μg chlorophyll/ml). Explanation in text.

Table I. *Light-Induced Structural Changes in* Euglena *Chloroplasts*

	Increased scattering (%)
Complete*	14
-$MgCl_2$ (5 mM)	5
-PMS (20 μM)	4
-Ascorbate (2 mM)	3
-Ascorbate, PMS	4
-Phosphate (2 mM)	14
Complete + DCMU (10 μM)	14
+ NH_4Cl (2 mM)	2
+ m-CCP (10 *μm*)	3
+ Antimycin *A* (2 μg/ml)	14
+ ADP (2 mM)	14
+ ATP (1 mM)	14

* Conditions for complete system as in figure 5. Explanation in text.

light-scattering changes at least 80 %, probably because it essentially caused the destruction of all the ascorbate present (see table I for ascorbate requirement).

Discussion

Light-induced conformational changes occur in *Euglena* chloroplasts in vitro, as seen from "chlorocrit" and light-scattering studies. Packed volumes were smaller and scattering increases larger upon illumination in the presence of appropriate cofactors, whereas NH_4Cl caused an opposite effect. Thus, in a general way, the factors which influence conformational changes in *Euglena* and spinach chloroplasts (1, 11, 12) are similar. However, the behavior of *Euglena* and of spinach chloroplasts and chromatophores of *Rhodospirillum rubrum* (14) differ with regard to the action of some cofactors. Mg^{++} and PMS are often required for maximal scattering responses, but *Euglena* chloroplasts and chromatophores also require ascorbate; exogenous phosphate is necessary in chromatophores, but not in *Euglena* chloroplasts. Some of these variations may result from different amounts of endogenous cofactors and the age of the chloroplasts.

Although it is clear that light-induced packed volume decreases occur in chloroplasts (after completion of sedimentation), the interpretation of these findings solely in terms of shrinkage is unwarranted without additional information. Itoh et al. (3) observed a decreased packed volume of 14 % in spinach chloroplasts in the light. This result qualitatively but not quantitatively agreed with their Coulter counter experiments (38 % shrinkage). The discrepancy was attributed to trapped space between the chloroplasts in the packed volume studies. In support of their suggestion, we have observed that centrifugation and pre-illumination time (fig 3, 4) in addition to shrinkage also affect the observed packed volume. When equilibrium is reached, the packed volume in the light is smaller than in the dark. It is of interest that photophosphorylation cofactors and inhibitors modify both the sedimentation pattern and the final packed volume. The unusual feature of these experiments is that the intensity and duration of light exposure change the pattern of sedimentation. How light brings this about is not yet known. However, it is interesting that Zurzycki (16), investigating the ability of chloroplasts to be displaced in vivo by centrifugation, has observed that the mobility of the chloroplasts was also dependent on intensity and illumination time. These changes were correlated with the shape of the chloroplast profiles.

Summary

Evidence for light-induced conformational changes has been demonstrated in *Euglena gracilis,* strain z, chloroplast preparations by packed volume or "chlorocrit" determinations and from light-scattering studies. Upon illumination the pattern of chloroplast sedimentation and packed volume is significantly different from dark controls. A packed volume decrease dependent on light intensity, pH, and temperature was observed. Cofactors and inhibitors of photophosphorylation also affected the "chlorocrit" determinations. Light-scattering increases induced by actinic red light required magnesium, ascorbate, and phenazine methosulfate, and are reversible, decaying in the dark.

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